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Application Number 10/604,340

***(A) Amendments to the Claims***

**Amendment 1: Claims 8, 10, 11, 21, 23, 24, 26, 27, 29, 36**

The authors would like to amend claims 8, 10, 11, 21, 23, 24, 26, 27, 29, 36 for the following reason: These claims are written as composition of matter claims, but are dependent on method claims. The patent officer was so kind as to point out that composition of matter claims cannot depend on method claims. Thus, the inventors would like to correct the claims in question and "transform" them into method claims. No new subject matter has been added.

**Amendment 2: Claim 36**

This claim is amended in response to <5> from the Office Action Summary of 6/17/2005. The inventors agree with the officer's assessment, i.e., that one (1) RNA target sequence is considered reasonable and thus limit the number of target sequences in claim 36 to one (VEGF). No new subject matter has been added.

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**CLAIMS**

We claim:

Claim 1 (original): A method of decreasing the expression of a target gene in a cell of a mammalian subject comprising administering to the subject in vivo a therapeutically effective amount of an RNAi expression cassette, comprising: (a) providing a recombinant adeno-associated viral vector, wherein said vector comprises said RNAi expression cassette whose RNA expression product(s) directly or indirectly lead to the decrease of expression of an RNAi target gene, wherein the RNA expression product(s) of the RNAi expression cassette comprise a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene mRNA transcript (b) delivering said recombinant adeno-associated viral vector to and/or within said mammalian subject wherein transduction of suitable target cells results in expression of said RNAi expression cassette.

Claim 2 (original): A method of decreasing the expression of (at least) one target gene in a cell of a mammalian subject comprising administering to the subject in vivo a therapeutically effective amount of (at least) one RNAi expression cassette, comprising: (a) providing (at least) one recombinant adeno-associated viral vector, wherein said vector comprises (at least) one RNAi expression cassette whose RNA expression product(s) directly or indirectly lead to the decrease of expression of an RNAi target gene, wherein the RNA expression product(s) of the RNAi expression cassette comprise a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene mRNA transcript (b) delivering said recombinant adeno-associated viral vector(s) to and/or within said mammalian subject wherein transduction of suitable target cells results in expression of said RNAi expression cassette.

Claim 3 (original): The method of claims 1 and 2, wherein expression of the RNA coding region of the RNAi expression cassette results in the down-regulation of the expression of the RNAi target gene, wherein the target gene comprises a sequence that is at least about 90% identical with the RNA coding region.

Claim 4 (original): The method of claims 1 and 2, in which the RNAi target gene expression is inhibited by at least 10%.

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Claim 5 (original): The method of claims 1 and 2, wherein said RNAi expression cassette(s) encode one or more RNA molecules which are capable of forming an RNA interference inducing double-stranded RNA complex.

Claim 6 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) one RNA molecule which is self-complementary.

Claim 7 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) two separate complementary single-stranded RNA molecules.

Claim 8 (currently amended): ~~The RNA molecule or RNA molecules of claims 6 and 7~~The method of claims 6 and 7, wherein said RNA molecule or RNA molecules are capable of forming an RNA interference inducing double-stranded RNA complex.

Claim 9 (original): The method of claims 1 and 2, wherein (at least) two recombinant adeno-associated viral vectors are used with each vector comprising its own RNAi expression cassette, and each RNAi expression cassette encoding at least one RNA molecule which is complementary to the RNA molecule expressed by the other RNAi expression cassette.

Claim 10 (currently amended): The method of claims 5, 6, 7, 8, and 9, where the~~The~~ RNA molecule(s) ~~of claims 6, 6, 7, 8 and 9 having~~ have a nucleotide sequence which is substantially identical and/or complementary to at least a part of the RNAi target gene.

Claim 11 (currently amended): The method ~~The RNA molecule(s)~~ of claims 5, 6, 7, 8 and 9 with the RNA molecule(s) being siRNA.

Claim 12 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes a self-complementary RNA molecule comprising a sense region, a loop region and an antisense region.

Claim 13 (original): The method of claim 12, wherein the loop region is about 2 to about 10 nucleotides in length.

Claim 14 (original): The method of claim 12, wherein the sense region and the antisense region are each between about 10 and about 30 nucleotides in length.

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Claim 15 (original): The method of claim 12, wherein the sense region hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene, and the antisense region, which is a complementary inverted repeat of said sense region, hybridizes to said sense region to form a hairpin structure.

Claim 16 (original): The method of claims 1 and 2, wherein said RNAi expression cassette comprises a first promoter and a second promoter, each operably linked to an RNA coding region, such that expression of the RNA coding region from the first promoter results in the synthesis of a first RNA molecule and expression of the RNA coding region from the second promoter results in the synthesis of a second RNA molecule substantially complementary to the first RNA molecule.

Claim 17 (original): The method of claims 1 and 2, wherein said RNAi expression cassette comprises two promoters operably linked to the same RNA coding region, such that expression of the RNA coding region from the first promoter results in the synthesis of a first RNA molecule and expression of the RNA coding region from the second promoter results in the synthesis of a second RNA molecule substantially complementary to the first RNA molecule.

Claim 18 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) two RNA molecules, wherein (a) one of the (at least) two RNA molecules consists essentially of a ribonucleotide sequence which corresponds to a nucleotide sequence of the RNAi target gene and another of the (at least) two RNA molecules consists essentially of a ribonucleotide sequence which is complementary to said nucleotide sequence of the RNAi target gene (b) the (at least) two RNA molecules are separate complementary strands that hybridize to each other to form a double-stranded RNA complex, and the double-stranded RNA complex directly or indirectly inhibits expression of the RNAi target gene.

Claim 19 (original): The method of claims 1 and 2, wherein said RNAi expression cassette comprises a promoter operably linked to a DNA sequence which, when expressed by a host cell produces one RNA molecule having: (a) homology to at least one target mRNA expressed by the host cell (b) two (internally) complementary RNA regions wherein the expressed RNA reduces the intracellular concentration of the target mRNA or any substantially similar endogenous mRNA either directly or indirectly.

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Claim 20 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes (at least) one RNA molecule for inhibiting expression of a target gene, comprising a first nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the RNAi target gene, and a second nucleotide sequence which is a complementary inverted repeat of said first nucleotide sequence and hybridizes to said first nucleotide sequence to form a hairpin structure.

Claim 21 (currently amended): The method ~~RNA molecule~~ of claim 19, wherein the two nucleotide sequences of said RNA molecule are joined by an RNA loop structure.

Claim 22 (original): The method of claims 1 and 2, wherein expression of said RNAi expression cassette leads to the generation of a double-stranded RNA complex comprising: (a) a first RNA portion capable of hybridizing under physiological conditions to at least a part of an mRNA molecule encoded by a gene; and (b) a second RNA portion wherein at least a part of the second RNA portion is capable of hybridizing under physiological conditions to the first RNA portion.

Claim 23 (currently amended): The method ~~The RNA complex~~ of claim 22, wherein the first and second portions of said RNA complex are separate ribonucleic acid molecules.

Claim 24 (currently amended): The method ~~RNA complex~~ of claim 22, wherein the first and second portions of said RNA complex are comprised within the same RNA molecule.

Claim 25 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes a linear RNA molecule capable of forming a double-stranded RNA complex wherein the RNA molecule comprises: (a) a first portion that hybridizes under physiologic conditions to at least a portion of an mRNA molecule encoded by a gene; and (b) a second portion wherein at least part of the second portion is capable of hybridizing to the first portion to form a hairpin double-stranded RNA complex.

Claim 26 (currently amended): The method ~~linear RNA molecule~~ of claim 25, wherein said linear RNA molecule further comprises ~~comprising~~ a third portion of ribonucleic acid interposed between the first and second portions.

Claim 27 (currently amended): The method ~~linear RNA molecule~~ of claim 26, wherein the third portion of said linear RNA molecule promotes hybridization between the first and second portion.

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Claim 28 (original): The method of claims 1 and 2, wherein said RNAi expression cassette encodes a linear RNA molecule capable of forming a double-stranded RNA complex wherein the RNA molecule comprises: (a) a first portion that comprises a region of RNA that is complementary to at least a portion of an mRNA molecule encoded by a gene (b) a second portion capable of hybridizing to at least part of the first portion (c) a third portion positioned between the first and second portions to facilitate the hybridization of the first and second portions with one another.

Claim 29 (currently amended): The method ~~linear RNA molecule~~ of claim 22 and 25, wherein the second sequence of said linear RNA molecule comprises a transcription termination signal positioned at the 3' end of the linear RNA molecule.

Claim 30 (original): The method of claims 1 and 2, wherein the recombinant adeno-associated viral vector further comprises a gene of interest.

Claim 31 (original): The method of claims 1 and 2, wherein the rAAV vector is of serotype 1, 2, 3, 4, 5, 6, 7 or 8 or any homologous serotypes or hybrids thereof.

Claim 32 (original): The method of claims 1 and 2, wherein said RNAi expression cassette comprises an RNA Polymerase III promoter.

Claim 33 (original): The method of claims 1 and 2, wherein said RNAi expression cassette comprises an RNA Polymerase II promoter.

Claim 34 (original): The method of claims 1 and 2, wherein said RNAi expression cassette comprises an RNA Polymerase I promoter.

Claim 35 (original): The method of claims 1 and 2, wherein said RNAi target gene causes or is likely to cause disease.

Claim 36 (currently amended): The method of claims 1 and 2, wherein said RNAi target genes ~~are the Rhodopsin gene, the CCR5 gene, the CXCR4 gene~~ is the VEGF gene~~, the HIF gene or any other gene of therapeutic interest~~.

Claim 37 (original): The method of claims 1 and 2, wherein said RNAi target gene is the Rhodopsin gene.

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Claim 38 (original): The method of claims 1 and 2, wherein said transduced cells are cells of and/or in the eye, retinal cells, retinal pigment epithelial cells, photoreceptor cells, cells of the eye, gut cells, muscle cells, lung cells, intestinal cells, liver cells, pancreatic cells, hematopoietic cells, stem cells, skin cells, endothelial cells, neurons, cells of ectodermal origin, cells of neurodermal original, cells of endodermal original and/or brain cells.

Claim 39 (original): The method of claims 1 and 2, wherein said transduced cells are photoreceptor cells.

Claim 40 (original): The pharmaceutical preparation comprising a recombinant adeno-associated viral vector comprising an RNAi expression cassette as claimed in claim 1.

Claim 41 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by intravenous administration.

Claim 42 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by intra-arterial administration.

Claim 43 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by intracavity injection.

Claim 44 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by injection into tissue.

Claim 45 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by injection into gaps in tissue.

Claim 46 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by local administration.

Claim 47 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by inhalation and/or nasal instillation.

Claim 48 (original): The pharmaceutical preparation as claimed in claim 40, wherein said preparation is suitable for and/or administered by intraocular and/or intravitreal administration.

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Claim 49 (original): A method for treating a mammalian subject with an autosomal-dominant disorder or other disease including cancer and infectious diseases byadministering to the subject an adeno-associated viral vector for initiating decrease of RNAI target gene expression at the mRNA level, wherein the method comprises using RNAi to achieve post-transcriptional gene silencing.

Claim 50 (original): The method of claim 49, wherein the mammalian subject is a human patient.